Saskatchewan
Agriculture, Food
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FINAL REPORT

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ANIMAL DISEASE DIAGNOSIS: DEVELOPMENT OF DIAGNOSTIC TESTS FOR INFECTIOUS AND NON-INFECTIOUS DISEASES OF LIVESTOCK

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FINAL PROJECT REPORT
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PROJECT TITLE
Animal Disease Diagnostics

PROJECT SUMMARY

The Animal Disease Diagnosis Research Project's objectives were to develop and validate new or improved diagnostic tests for animal diseases that are needed by livestock industry in Saskatchewan. New tests were developed with priorities set through regular consultation with the livestock industry, the veterinary profession and the WCVM's Disease investigation Unit. Work was be carried out for infectious diseases and non-infectious disease diagnosis. Screening assessments of unusual or diagnostically-challenging outbreaks of disease were also done to gather information for early recognition of new disease problems for which new test development might be required. The project does not itself, provide a testing or diagnostic service. Validated tests have been transferred to Prairie Diagnostic Services (PDS) which provides diagnostic services to agricultural producers.

Resulting from this research are validated diagnostic test procedures which can be immediately implemented by PDS and other diagnostic service companies. Documentation of sensitivity, specificity, detection limit, predictive value of positive and negative tests are all crucial to the critical evaluation of any new test. Interpretation of diagnostic results, herd and animal health status, treatment interventions, and conformation with international trade rules are dependent on valid and accurate test results which this project will provide.

SUMMARY

Pathogen	Proposed Start date	Completion date	Status
Newcastle Disease Virus	May 2003	April 2004	Done
Cyanobacterial toxins	July 2003	December 2003	Cancelled
Equine Influenza	already started	August 2003	Done
Swine Erysipelas	already started	August 2004	Done
A rapid test for BVD Virus	July 2003	April 2004	Done
Mycoplasma synoviae	already started	April 2004	Done
Mycoplasma bovis	September 2003	April 2004	Done
Ureaplasma sp.	September 2003	October 2004	On going

Introduction:

In early January 2003 a meeting of the Diagnostic Test-Development Advisory Group was held to set test-development priorities for the coming year. Members of this committee consisted of representatives of PDS, the departments of Veterinary Microbiology, Veterinary Pathology and the Disease Investigation Unit of the Western College of Veterinary Medicine. Suggestions received from the veterinary and livestock industries over the past year were included. The list of disease-causing agents for which tests will be developed, reflected the guidance given. The primary requirement for validation is that we obtain positive and negative samples to provide a statistically valid assessment of the assay's function.

Newcastle Disease Virus

Newcastle Disease is a contagious and fatal viral disease affecting most species of birds. Clinical signs are extremely variable depending on the strain of virus, species and age of bird, concurrent disease, and preexisting immunity. Some strains of NDV is so virulent that many birds die without showing any clinical signs and a death rate of almost 100 percent can occur in unvaccinated poultry flocks. On many occasions in the previous 10 years, mortality due to the Newcastle virus has been observed in waterfowl in Manitoba and Saskatchewan. A PCR assay will provide the capability to identify the virus rapidly and accurately. This assay has been optimized using positive control material from wild birds as well as virus obtained from CFIA - Winnipeg.

Cyanobacterial toxins

Detection of toxin produced by blue-green algae (Cyanobacteria) is still a priority and when a field test kit becomes available from researchers at the University of Alberta and we will evaluate it for use in Saskatchewan. We have discontinued work on this project because of delays in obtaining the field-kit.

Equine Influenza (detection of type A and H7/H3 typing)

Influenza virus is one of the most common causes of respiratory disease in horses. It is extremely contagious and can predispose horses to serious secondary bacterial infections of the respiratory tract. There is an effective vaccine program for Equine Influenza but strain typing is critical to the success of that program. Virus detection and subsequent typing by molecular assay will rapidly provide the information needed to initiate effective control. This assay has been optimized using positive control material from a recent local outbreak.

Swine Erysipelas

Acute infections with *Erysipelothrix rhusiopathiae* can be diagnosed through pathological presentation and standard bacterial culturing methods but the chronic form, often seen three or more weeks after the initial infection, is more difficult and low numbers of organism makes bacterial culture less reliable. A molecular test with high sensitivity will offer benefits over current practices. This assay has been optimized and is being validated with the cooperation of a local swine-specialist veterinarian.

A rapid test for BVD Virus

From the economic standpoint, bovine viral diarrhea (BVD) is one of the most important diseases of cattle in the North America. There is an urgent need for a rapid diagnostic

assay for large scale screening and removal of BVD virus-infected cattle. Issues to consider when developing a rapid test include the degree to which the samples can be pooled, the cost, accuracy and speed. This assay has been optimized and validated using field-derived material from a large BVD survey conducted by a feedlot management company in Alberta. A pooling strategy has been employed which minimizes cost without sacrificing too much sensitivity. An extraction protocol has been adapted which can work using whole blood. Validation of this assay is being performed by comparison with immunohistochemistry on paired-skin biopsy samples.

Mycoplasma synoviae

Chronic respiratory disease caused by *Mycoplasma synoviae* infection occurs world-wide and respiratory disease is responsible for extensive losses in broiler operations especially where concurrent viral respiratory diseases occur. Mycoplasma spp can be isolated and identified by inoculating tracheal swabs or serous joint exudate from acute cases onto special selective media but this procedure may require up to 30 days and may be inconclusive due to technical problems including contamination. The polymerase chain reaction can be applied with the additional goal of distinguishing *Mycoplasma synoviae* from *Mycoplasma gallicepticum*. This assay has been optimized.

Mycoplasma bovis

Bovine diseases due to *Mycoplasma bovis* can cause considerable economic losses in cattle production. While the pathogen is principally responsible for therapy-resistant mastitis on large dairy farms, on smaller farms the typical mycoplasma diseases are calf pneumonia and arthritis. *M. bovis* infection can be controlled effectively only if appropriate measures are implemented at the earliest possible stage. Frequently, testing for *Mycoplasma* is not included in routine bacterial examination of clinical samples and, as a consequence, some *M. bovis* infections remain undetected and outbreaks cannot be controlled properly. This assay has been optimized.

***Ureaplasma* sp.**

This pathogen plays a role in urogenital and reproductive disease in a number of livestock species and may be involved in bovine abortions. An accurate test that does not require culture has the potential to improve diagnosis of causes of abortion. Optimization of this assay is ongoing.

Additional investigations and projects

Due to our expertise in identifying organisms for which no other diagnostic assay is available, we have been approached by various researchers across Canada to assist with pathogen identification. We are currently working with CFIA to differentiate *Campylobacter fetus fetus* from *Campylobacter fetus venerealis*. We are working with researchers at the Western College of Veterinary Medicine to identify fowlpox virus, avian adenovirus, equine herpesvirus, *Bartonella* sp., *Mycoplasma* sp and *Blastomyces* sp. We also worked with researchers in Ontario and PEI to identify species of *Mycobacterium* in selected cases of unexplained illness.

Repertoire of PCR tests for Animal Pathogens (ADF supported research 1997-2003)

Agent

Aeromonas salmonicidia
Ascaris lumbricoides
Ascaris suum
Bacillus sp.
Bacillus anthracis
Bartonella sp.
Blastomyces sp.
Bovine respiratory syncytial virus
Bovine herpesvirus
Bovine papillomavirus
Bovine rotavirus
Bovine coronavirus
Bovine viral diarrhea virus
Brachyspira innocens,
Brachyspira pilisicoli
Brachyspira hyodysenteriae
Brachispira murdochii
Brachispira intermedia
Campylobacter fetus fetus
Campylobacter fetus venerealis
Campylobacter sp
Canine parvovirus
Chitrid fungus
Chlamydia sp.
Chlamydia psittici
Clostridium botulinum
Clostridium difficile
Clostridium perfringens
Clostridium piliformis
Coronavirus (SARS)
Coxiella burnetti
Cryptosporidium sp.
Cryptosporidium parvum
Cryptosporidium canis
Cryptosporidium felis
Echinococcus sp
Eimeria sp
Elaphostrongylus sp
Enterococcus sp
Equine herpesvirus
Equine papillomavirus
Erysipelothrix rhusiopathiae
Escherichia coli
Fowl adenovirus
Fowl pox
Francisella tularensis
Fusobacterium necrophorum
Giardia sp.
Herpesviruses (pan, cervid, CHV, AHV, OHV, BLH, equine)
Influenza (A, avian H1, H5, H7, equine H1, H3, swine H1, H3)
Iridovirus
Isospora suis
Lawsonia intracellularis
Listeria monocytogenese
Monkeypox
Mycobacterium avium paratuberculosis
Mycobacterium avium avium
Mycobacterium sp.
Mycobacterium bovis
Mycoplasma sp
Mycoplasma synoviae
Mycoplasma mycoides mycoides
Mycoplasma hyopneumoniae
Mycoplasma capricolum capripneumoniae
Mycoplasma bovis
Mycoplasma gallisepticum
Mycoplasma haemocanis
Mycoplasma haemofelis
Mycoplasma haemominutum
Mycoplasma haemolama
Mycoplasma hyosynoviae
Myxobolus cerebralis
Neospora caninum
Newcastle Disease Virus
Parascaris equorum
Parelaphostrongylus sp
Pasteurella multocida
Pneumocystis sp.
Porcine circovirus 1
Porcine circovirus 2
Propionibacterium sp
PRRS Virus
Ranavirus
Reimerella anatipestifer
Renibacterium salmoninarum
Salmonella enterica subsp
Staphylococcus aureus
Streptococcus equi
Toxoplasma gondii
Ureaplasma sp.
Vibrio vulnificus
Varestrongylus sp.
West Nile Virus
Yersinia pestis
Yersinia pestis
Yersinia ruckeri
Yersinia enterocolitica
Yersinia pseudotuberculosis

Secondary Outputs:

PAPERS IN REFEREED JOURNALS:

J.A. Berezowski, **Appleyard, G.D.**, T. Crawford, J. Haigh, H. Li, D. Middleton, B. O'Connor, K. West, & M Woodbury. Epidemiology of a severe outbreak of sheep associated malignant catarrhal fever in bison (*Bison bison*) following brief exposure to sheep at a public auction sale. Accepted by J of Vet Diagnostic Investigation 2003.

L.B. Forbes, **Appleyard, G.D.** & A.A. Gajadhar. Comparison of an ELISA using synthetic tyvelose antigen with an ELISA using excretory-secretory antigen for the detection of trichinellosis in swine. Accepted by Journal of Parasitology 2003.

K.E. Kewish, **Appleyard, G.D.**, S Meyers, B.A. Kidney & ML Jackson. *Mycoplasma haemofelis* and *Mycoplasma haemominutum* detection by polymerase chain reaction in cats from Saskatchewan and Alberta. Accepted by Can Vet J 2003.

SL Checkley, CL Waldner, **GD Appleyard**, JR Campbell, ED Janzen. The Comparison of 5 diagnostic tests for diagnosis of Johne's Disease in Western Canada. Journal of Applied Research in Veterinary Medicine, Vol 1, no 3, Fall 2003.

J. Sibley, B. Yue, F. Huang, J. Harding, J. Kingdon, M. Chirino-Trejo & **G.D. Appleyard**. Comparison of bacterial enrichment-broth culture, ELISA and PCR techniques for identifying the shedding of *Salmonella* from asymptomatic swine. Canadian Journal of Veterinary Research, 2003, 67, 219-224.

M. Ngeleka, J.M. Fairbrother, J. Pritchard, **G.D. Appleyard** & D. Middleton. *Escherichia coli* diarrhea in piglets: isolation and association of AIDA-I/STb, rather than EAST1 pathotype with diseased animals and antibiotic sensitivity of isolates. Journal of Veterinary Diagnostic Investigations, 2003, 15: 242-252.

INVITED LECTURES AND INVITED CONFERENCE PRESENTATIONS:

G.D. Appleyard, Animals Disease Diagnostics and Pathogen Discovery. Virtual College of Biotechnology. University of Saskatchewan. January 2004.

E. Jenkins, A.M. Veitch, **G.D. Appleyard**, E.P. Hoberg, S.J. Kutz, and L. Polley. Geographic Distribution and Seasonal Patterns of Larval Shedding of the Muscle-Dwelling Nematode Parelaphostrongylus odocoilei in Thinhorn Sheep from Northern North America. ARCUS 15th Annual Meeting and Arctic Forum 2003.

PRESENTATIONS AT CONFERENCES

Emily Jenkins, Alasdair Veitch, **Greg Appleyard**, Eric Hoberg, Dele Ogunremi, Susan Kutz, Lydden Polley. A newly recognized neurological disease associated with *Parelaphostrongylus odocoilei* in experimentally infected thinhorn sheep. 53rd Annual Meeting of the Wildlife Disease Association. August, 2004. San Diego, CA

Susan Kutz, **Greg Appleyard**, Emily Jenkins, Aedes Scheer, John Nagy, Alasdair Veitch, Brett Elkin, Dorothy Cooley, Deborah Johnson, Eric Hoberg, Lydden Polley. Assessing, Predicting, and Monitoring the Impact of Climate Change on Infectious Diseases in Caribou: What Do We Know and What Do We Need to Know? 10th North American Caribou Conference. Alaska. May 2004.

K. Kewish, **G.D. Appleyard**, S. Meyers, B. Kidney and M. Jackson. PCR analysis for Mycoplasma haemofelis and "Candidatus Mycoplasma haemominutum" in cats from Saskatchewan and Alberta. American College of Veterinary Pathologists Conference, Lake Louise, Nov 2003.

J.L. Davies, J.A. Sibley, E.G. Clark, and **G.D. Appleyard**. Feline leprosy syndrome is associated with several mycobacterium species: a histologiuc and genotypic retrospective study on formalin fixed and paraffin embedded tissues. American College of Veterinary Pathologists Conference, Lake Louise, Nov 2003.

C. Jardine, **G.D. Appleyard**, M Chirino-Trejo, F.A. Leighton and G Wobeser. Wild rodent associated Bartonella: a group of new and emerging bacteria in Saskatchewan, Canada. Wildlife Disease Association Conference, Aug Saskatoon, 2003.

J.L. Davies, J.A. Sibley, E.G. Clark, and **G.D. Appleyard**. Histologic and genotypic characterization of cutaneous mycobacteriosis in cats: a retrospective study on formalin-fixed and paraffin embedded tissues. American Association of Veterinary Laboratory Diagnosticians, San Diego, Oct 2003.

J.A. Sibley and **G.D. Appleyard**. Detection of selected wildlife pathogens in commercial waste digestion products containing bacteria. American Association of Veterinary Laboratory Diagnosticians, San Diego, Oct 2003.

K.A. McIntosh, J.C.S. Harding, J.A. Ellis, and **G.D. Appleyard**. Detection of porcine circovirus type 2 viremia and seroconversion in swine. American Association of Veterinary Laboratory Diagnosticians, San Diego, Oct 2003.

S. Kutz, E. Jenkins, E. Hoberg, **GD Appleyard**, A. Veitch, J. Nagy, B. Elkin, J. Nishi, R. Mulders, B. Rosenthal, and L. Polley. Ungulate Parasitology in the Canadian North. Second International Workshop on Arctic Parasitology (IWAP 2), Rovaniemi, Finland, Sept. 1-3, 2003.

Susan Kutz, Emily Jenkins, Eric Hoberg, **Greg Appleyard**, Alasdair Veitch, John Nagy, John Nishi, Brett Elkin, Robert Mulders, Ben Rosenthal & Lydden Polley. Ungulate parasitology in the Canadian north. 11th Arctic Ungulate Conference, Saariselkä, Finland, 2003

Meiqing Shi, Wanling Pan, Guojian Wei, **Greg Appleyard**, Chyung-Ru Wang & Henry Tabel CD1-restricted antigen presentation does not play a major role in the course of infection of mice infected with *Trypanosoma congolense* or *T. brucei*. Canadian Society for Immunology, 2003.